TMH/DAG:jlb 08/26/02 136209.doc PATENT

candd:

was detected using the DELFIATM system, the downstream primer was biotinylated at the 5' end to allow specific capture of amplified sequences through the use of streptavidin.

Please replace the paragraph at page 28, lines 3-13 with the following:

Ć10

Southern Blotting: Standard southern blotting techniques were used to confirm the PCR results (Tables 2 and 3). Following agarose gel electrophoresis, PCR products were transferred to Hybond N+ membranes (Amersham, Live Science, Arlington Heights, IL). Amplification of human-*P. carinii MSG* was detected using probe JKK16 (SEQ ID NO: 19), which corresponds to residues of 2926-2950 of *HMSG33*. Amplification of *P. carinii* MRSU was detected using pAZ102-L2 (Wakefield *et al.* (1990) *Mol. and Biochem. Parasitol.* 43:69-76). Oligonucleotides were labeled with [γ-³²P]-ATP by T4 polynucleotide kinase (Ready-to-GoTM Molecular Biology Reagents, Pharmacia Biotech, Denmark). Prehybridization and hybridization were performed overnight at 52° C in 6 X SSPE, 1% sodium dodecyl sulfate (SDS), 10 X Denhardts' solution (Research Genetics, Huntsville, Alabama). Filters were washed at 52° C in 1 x SSPE, 0.5% SDS for 30 min, then 0.1 x SSPE, 0.5% SDS for 15 minutes.

In the Claims:

Please amend the claims to read as follows:

CH

1. (amended) A method of detecting the presence of *Pneumocystis carinii* in a human biological specimen, comprising:

amplifying a highly conserved region within a human-*P. carinii* nucleic acid sequence, if such sequence is present in the specimen, using two or more oligonucleotide primers that hybridize to the highly conserved region; and

determining whether an amplified sequence is present,
wherein the highly conserved region has at least 79% sequence identity with residues 2794-3042
of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11*(SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID
NO: 9), 2809-3054 of *HMSG33* (SEQ ID NO: 11), or 1-249 of *HMSGp2* (SEQ ID NO: 15); or at least 84% sequence identity with residues 2821-3072 of *HMSG35* (SEQ ID NO: 13); and wherein at least one oligonucleotide primer hybridizes to residues 2794-2886 of *HMSGp1* (SEQ

TMH/DAG:jlb 08/26/02 136209.doc PATENT

conclet

ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

- 2. (reiterated) The method according to claim 1, wherein amplification of the human-*P. carinii* nucleic acid sequence is by polymerase chain reaction.
- C12
- 3. (twice amended) The method of claim 1, wherein the oligonucleotide primers hybridize under low stringency conditions comprising 50°C in 6x SSC, 5x Denhardt's solution, 0.5% SDS and 100 µg sheared salmon testes DNA.
- 4. (twice amended) The method of claim 1, wherein the highly conserved region comprises a sequence selected from the group consisting of: residues 2794-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2809-3054 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).
- 5. (**twice amended**) The method of claim 1, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).
- 6. (twice amended) The method of claim 1, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides having at least 91% sequence homology with approximately the same number of nucleotides of residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of

TMH/DAG:jlb 08/26/02 136209.doc PATENT

HMSG33 (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

7. (**twice amended**) The method of claim 1, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides having at least 95% sequence homology with approximately the same number of nucleotides of residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

- 8. (amended) The method of claim 1, wherein the oligonucleotide primers hybridize under stringent conditions comprising 65°C in 6x SSC, 5x Denhardt's solution, 0.5% SDS and 100 µg sheared salmon testes DNA.
 - 9. (amended) The method of claim 1, wherein the oligonucleotide primers consist of one upstream primer and one downstream primer.
 - 10. (amended) The method of claim 9, wherein: the upstream primer is SEQ ID NO: 17, or SEQ ID NO: 18; and the downstream primer is SEQ ID NO: 20 or SEQ ID NO: 24.
 - 11. (amended) The method of claim 1, wherein one of the oligonucleotide primers comprises SEQ ID NO: 17.
 - 12. (amended) The method of claim 1, wherein one of the oligonucleotide primers comprises SEQ ID NO: 18.
 - 13. (amended) The method of claim 1, wherein one of the oligonucleotide primers comprises SEQ ID NO: 19.

CB Jel

14. (amended) The method of claim 1, wherein one of the oligonucleotide primers comprises SEQ ID NO: 20.

15. Please cancel claim 15.

UL

- 16. (amended) The method of claim 1, wherein one of the oligonucleotide primers comprises SEQ ID NO: 24.
- 17. (amended) The method of claim 1, wherein the specimen is from the oropharyngeal tract.
 - 18. (amended) The method of claim 1, wherein the specimen is from blood.
- 19. (reiterated) The method of claim 1, wherein the step of determining whether an amplified equence is present comprises one or more of:
 - (a) electrophoresis and staining of the amplified sequence; or
 - (b) hybridization to a labeled probe of the amplified sequence.
- 20. (**reiterated**) The method of claim 19, wherein the amplified sequence is detected by hybridization to a labeled probe.

CIS

- 21. (amended) The method of claim 20, wherein the labeled probe comprises a detectable non-isotopic label chosen from the group consisting of:
 - a fluorescent molecule;
 - a chemiluminescent molecule;
 - an enzyme;
 - a co-factor;
 - an enzyme substrate; and
 - a hapten.

CP Del 2 CP NO: 19.

- 22. (amended) The method of claim 20, wherein the labeled probe comprises SEQ ID
- 23. (amended) A method of detecting the presence of *Pneumocystis carinii* in a human biological specimen, comprising:

exposing the specimen to a probe that hybridizes under stringent conditions to a human-*P. carinii* nucleic acid sequence, if the sequence is present in the specimen, to form a hybridization complex; and

determining whether the hybridization complex is present,

wherein the human-*P. carinii* nucleic acid sequence is *HMSGp1* (SEQ ID NO: 1), *HMSGp3* (SEQ ID NO: 3), *HMSG11* (SEQ ID NO: 5), *HMSG14* (SEQ ID NO: 7), *HMSG32* (SEQ ID NO: 9), *HMSG33* (SEQ ID NO: 11), *HMSG35* (SEQ ID NO: 13), or *HMSGp2* (SEQ ID NO: 15); and

wherein the stringent conditions of hybridization comprise 65°C in 6x SSC, 5x Denhardt's solution, 0.5% SDS and 100 µg sheared salmon testes DNA.

24. (amended) The method of claim 23, wherein the probe comprises SEQ ID NO: 19.

Please add the following new claims:

425. (new) The method of claim 23, wherein the probe is a labeled probe.

(new) The method of claim 1, wherein two or more of the oligonucleotide primers each comprise at least 15 contiguous nucleotides having at least 91% sequence homology with approximately the same number of nucleotides of residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

(new) The method of claim 1, wherein two or more of the oligonucleotide primers each comprise at least 15 contiguous nucleotides having at least 95% sequence homology with

and

approximately the same number of nucleotides of residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

(new) The method of claim 1, wherein two or more oligonucleotide primers each comprise at least 15 contiguous nucleotides from residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

(new) The method of claim 4, wherein two or more of the oligonucleotide primers hybridize to residues 2794-2886 of *HMSGp1* (SEQ ID NO: 1), 2758-2850 of *HMSGp3* (SEQ ID NO: 3), 2845-2937 of *HMSG11* (SEQ ID NO: 5), 2839-2931 of *HMSG14* (SEQ ID NO: 7), 2836-2928 of *HMSG32* (SEQ ID NO: 9), 2809-2901 of *HMSG33* (SEQ ID NO: 11), 2821-2913 of *HMSG35* (SEQ ID NO: 13), or 1-93 of *HMSGp2* (SEQ ID NO: 15).

30. (new) The method of claim 29, wherein the oligonucleotide primers hybridize under low stringency conditions comprising 50°C in 6x SSC, 5x Denhardt's solution, 0.5% SDS and 100 µg sheared salmon testes DNA.

31. (new) The method of claim 29, wherein the oligonucleotide primers hybridize under stringent conditions comprising 65°C in 6x SSC, 5x Denhardt's solution, 0.5% SDS and 100 µg sheared salmon testes DNA.

32. (new) A method of detecting the presence of *Pneumocystis carinii* in a human biological specimen, comprising: